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Methodical Basis for Analysis of Monosaccharide Profile of the Polysaccharide Complex in the Mixture Herbal Product (Pectorales Species No 2)

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ABSTRACT

Background: Mucolytic and expectorant drugs are important elements in the pharmacotherapy of respiratory diseases. Phytopectol No 2 (Pectorales species No 2) is one of the herbal multi-component drugs (herbal teas). Pectorales species No 2 (PS No 2) is a mixture of three types of medicinal plants presented on the Russian pharmaceutical market. It consists of coltsfoot leaves (40%), plantain leaves (30%), and licorice roots (30%). The PS No 2 infusion is a dosage form prepared from crushed or powdered plant material. The PS No 2 infusion exhibits expectorant and anti-inflammatory activity; it is used in the treatment of respiratory diseases and to facilitate the release of sputum.

Purpose: This review study aims to collect and analyze the literature data concerning the composition and content of polysaccharides, their monosaccharide composition in PS No 2 and its components.

Methods: We used different scientific databases such as PubMed, Scopus, Web of Science, e-Library, Google Scholar for information search.

Result: Nowadays, synthetic drugs, herbal remedies, and combined medicines with expectorant action are presented on the Russian and world markets. Herbal medicines can be classified into single drugs containing one plant/component, and medicines with two or more plants/components. Herbal medicines take leading places in the segment of the mucoactive drugs market along with synthetic ones in the Russian Federation. Most of the PS No 2 polysaccharides are arabinogalactans, rhamnogalacturonans, galacturonans, galactans. The arabinose (up to 15%), glucose (up to 26%), galactose (up to 23%), rhamnose (up to 40%), as well as galacturonic acid (up to 26%) are presented in monosaccharide composition of PS No 2 polysaccharide complex.

Conclusion: Polysaccharides of various types are the main group of biologically active compounds (BAC) in PS No 2. The monomer composition of the polysaccharides causing the pharmacological action of PS No 2 should be established to improve the standardization procedures of crude herbal drugs and to identify BAC. The ratio of these carbohydrates can serve as a marker parameter in the standardization of PS No 2.

Keywords: coltsfoot leaves, plantain leaves, licorice roots, Pectorales species No 2, polysaccharides, monosaccharides

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INTRODUCTION

Phytotherapy with herbal medicines is one of the methods that have proven itself due to the centuries-old practice in the treatment of various diseases. High safety profile, relatively low cost of herbal drugs, rich historical and clinical experience of use are the advantages of phytotherapy.

The share of herbal drugs was 25% in the Russian pharmaceutical market in 2017. The distribution diagram of the herbal drugs segment is shown in Figure 1 [1]. The sales of mucoactive drugs amounted to 9.2 billion rubles at final consumption prices or 49.7 million packages in the Russian pharmaceutical market for the first half of 2019 (according to IQVIA). Synthetic drugs held a leading position in this market segment. The herbal remedies were placed in the second position. A growing trend in the value of herbal drug sales was observed in 2019. This group showed a positive trend for 6 months of 2019 compared to the same period in 2018. The growth trend in sales of herbal drugs is shown in Figure 2 [2].

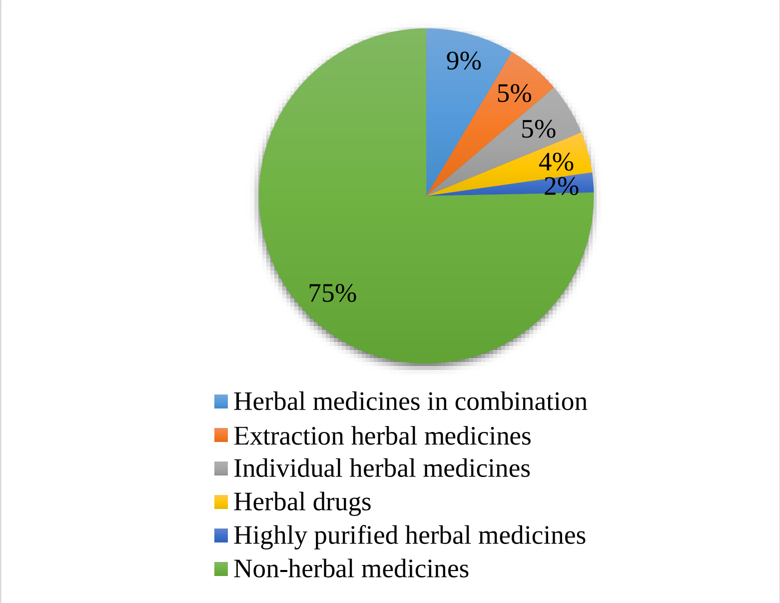

Figure 1: Share and structure of the segment of herbal medicines in the Russian pharmaceutical market (%)

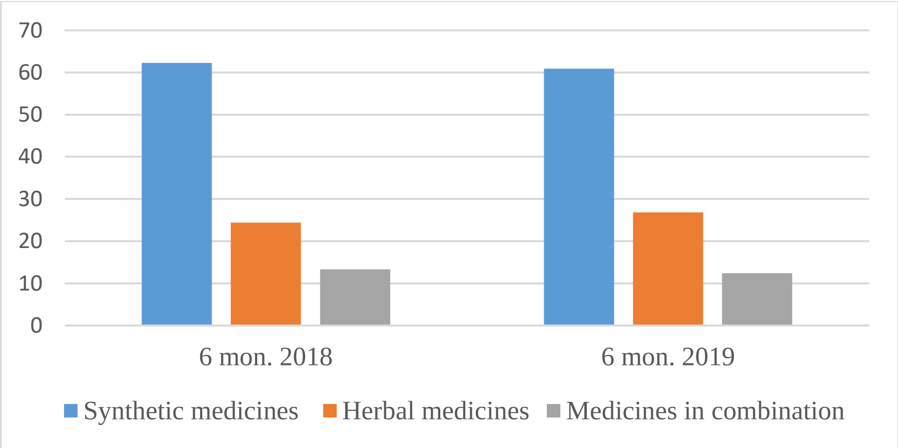

Figure 2: The ratio of the group of mucoactive drugs depending on the origin of the active components according to the results of 6 months (2018 and 2019), %.

Herbal expectorant drugs are one of the popular groups among all herbal medicines. The adverse effects of anthropogenic factors (industrial gas emissions), household allergens, plant pollen, and respiratory viral infections affect the formation and development of the pathology of respiratory diseases. Diseases of the upper and lower respiratory tract make up one-third of all outpatient visits to general practitioners [3]. Herbal expectorant drugs are presented as single drugs of various dosage forms (licorice syrup, plantain leaves, Mukaltin), and mixtures (herbal teas [4]) of several types of medicinal plant materials (Pectorales species). PS No 2 includes the coltsfoot leaves (Folia *Tussilaginis farfarae*) – 40%, large plantain leaves (Folia *Plantaginis majoris*) – 30%, licorice roots (Radices *Glycyrrhizae glabrae*) – 30%. The marketable forms of this medicine are crushed and powder (sachets) forms [5]. External signs of PS No 2 are shown in Figure 3.

Figure 3: External signs of Pectorales species No 2.

Medical applications for PS No 2 are inflammatory diseases of the respiratory tract accompanied by cough with sputum that is difficult to separate. The leaves of the coltsfoot contain polysaccharides – mucus (5-10%), inulin, dextrin, bitter glycosides, sitosterol, saponins, organic acids, ascorbic acid flavonoids (rutin, hyperoside), essential oils (karyofillen, α-cadinol, nerolidol), pyrrolizidine alkaloids (senkirkin and tussilagin). Plantain leaves contain polysaccharides, including mucus (up to 11%), iridoid glycosides (aucubin, catalpole), flavonoids (derivatives of luteolin, apigenin, scutellarein, baicalein), some tannins, carotenoids, ascorbic acid, vitamin K. Licorice roots contain glycyrrhizin (triterpene saponin, up to 23%), flavonoids, polysaccharides (starch, pectin substances) [6-10]. It is noteworthy that the chemical profile of each component of the PS No 2 includes polysaccharides, a group of compounds responsible for expectorant pharmacological action. The antitussive activity of mucus and pectin was established in [11]. In addition to the expectorant effect, plant polysaccharides exhibit antitumor, immunostimulating, anti-complementary, anti-inflammatory, antioxidant, anticoagulant, and fibrinogenic activity [12].

Identification of the polysaccharides type, chemical structure of monomers, and the ratio of monomers in polysaccharides are important aspects in the PS No 2 standardization. PS No 2 polysaccharides are the main BAC group and exhibit expectorant activity. This review study aims to collect and analyze literature data concerning the composition and content of polysaccharides and their monosaccharides in the PS No 2 components.

MATERIALS AND METHODS

2.1. Information search

We searched for the necessary information in various sources of scientific literature – electronic databases: PubMed, Google Scholar, Scopus, Web of Science, e-Library.

RESULTS AND DISCUSSION

3.1. Monosaccharide composition of the polysaccharide complex in the plantain leaves

Total polysaccharide content (determined by gravimetric procedure) should be not less than 12% according to State Pharmacopoeia of the Russian Federation XIV edition (SPRF XIV) [13]. Free carbohydrates of *Plantago major* L. (Folia *Plantaginis majoris*) leaves are represented by 9 monosaccharides: glucose (Glc), fructose (Frc), xylose (Xyl), rhamnose (Rha), raffinose (Raf) (0.03%), stachyose (Stc) (0.45%), galacturonic acid (GalUA), GalUA oligomers [14].

Gorin A.G. performed acid hydrolysis of a complex of water-soluble polysaccharides (yield 10%; ash content 28%). Nine monosaccharides have been determined; six monosaccharides were identified as GalUA, galactose (Gal), Ara, Rha, Glc, Xyl in trace amounts). The structure of the three monosaccharides has not been identified. Monosaccharide components were GalUA: Gal: Ara: Rha in the ratio of 16: 3: 2: 1 established after quantification [15].

The polysaccharide complex was separated on diethylaminoethyl cellulose (DEAE-C). It was found that its composition is represented by pectic acid (80-82%) mixed with arabinogalactan (5-6%) and galactan (4-5%). Enzymatic hydrolysis of pectic acid showed the presence of α-D-galacturonic acid. α-D-galacturonic acid is linked by 1,4 bonds [16].

Researchers in the Institute of Pharmacy (Norway), the Oriental Medicine Research Center of the Kitasato Institute (Japan) and the Cancer Research Institute (Norway) found that the neutral components are glucomannans- Glc 41.3-53.8% and mannose (Man) 11.7-36.25%. One of the acidic fractions is an arabinogalactan complex and mainly includes residues Ara (12.35-23.85%), Gal (18.1-34.05), Xyl (11.45-22.2%), insignificant the amount of Rha (3.0%). The second acid polysaccharide fraction was pectin containing 71.7% GalUA, 4.2% Rha, 8.8% Ara and 8% Gal. Fresh leaves collected in Oslo were stored in 80% ethanol to remove low molecular weight compounds. Then, extraction was performed with hot water (50 °C and100 °C), and dimethyl sulfoxide (DMSO). Separation into acidic and neutral fractions was carried out using ion-exchange chromatography on a DEAE-sepharose column with chloride counterion. Quantitative analysis was performed by gas chromatography (GS) with a flame ionization detector (PID). Preliminarily, polysaccharide samples were subjected to methanolysis with 4 M HCl in anhydrous methanol [17].

The pectin structure of plantain leaves was determined. A high degree of pectin esterification (68%) was detected. Every fifth GalUA residue bound by 1,4-glycosidic bonds is O-acylated. There are at least two branched sites represented by arabinogalactans. The more branched section consists mainly of Ara and Gal with a relatively high content of bonds 1→4 and 1→3,6 between them. It is bonded to the C4 atom of the Rha main chain. The second site is represented by Ara residues bounded with the C3 GalUA of the main chain. The ratio of GalUA: Gal: Ara: Rha in pectin is defined as 17: 2: 2: 1. The molecular weight is 46–48 kDa [18].

The ratio of Gal: Ara: Rha in the arabinogalactan component of the polysaccharide complex (molecular weight of 77-80 kDa) – 8: 6: 1. It consists of 1,3 bounded galactan in the main chain and 1,6 bounded arabinogalactan in the side branches (C6 attached). Additionally, there is the presence of 1→3 linked Gal residues in the side branches. They are bounded with 1→6 Gal, forming a network structure. The Ara residues in furanose form are bounded with galactan mainly at 1→6 linked (C3 attached) Gal side branches [19].

Researchers in the Medical University in Plovdiv (Bulgaria) determined the correlation between the structure of water-soluble polysaccharides of plantain leaves and the results of their enzymatic hydrolysis using various types of hydrolases. Galactose was identified as the main product of enzymatic hydrolysis. Type II arabinogalactans linked by β-1,3 or β-1,6-bonds and 1,4-linked pectin polysaccharides are believed to be major polysaccharides. Leaves were collected in May in the Plovdiv region. Then, extraction was performed with distilled water (1:25) in a boiling water bath. Water-soluble polysaccharides were precipitated with 95% alcohol. Hydrolysis was carried out using hemicellulase and mannanase enzymes. The monosaccharide composition of a 1% solution of water-soluble polysaccharides was determined by reverse phase high-performance liquid chromatography (HPLC) with a refractometric detector (quartz column; water mobile phase) [20].

The researchers also determined the quantitative content of monosaccharide residues of water-soluble and acid fractions of plantain leaves polysaccharides. The water-extractable polysaccharide fraction contained 37.36% Ara, 62.64% GalUA, and trace amounts of Rha. The acid polysaccharides included 16.96% Rha, 46.21% Gal, 36.93% GalUA, 2.18% non-hydrolyzed GalUA / Rha. The leaves were collected during the growing season in the Thracian floristic region. The polysaccharides were extracted from the leaves with water and dilute hydrochloric acid. Hydrolysis of acidic and water-soluble polysaccharides was carried out using 2M trifluoroacetic acid (TFA). The monosaccharide composition was determined by reverse phase HPLC with a refractometric detector (quartz column; water mobile phase) [21].

Data on the monosaccharide profile of the polysaccharide complex in plantain leaves are summarized in Table 1.

Table 1: Monosaccharide composition of the polysaccharide complex in the plantain leaves

Monosaccharide	Content, %
Arabinose (Ara)	8,8-37,36
Glucose (Glc)	41,3-53,8
Mannose (Man)	11,7-36,25
Galactose (Gal)	8-46,11
Rhamnose (Rha)	3,3-16,96
Xylose (Xyl)	11,45-22,22
Galacturonic acid (GalUA)	36,93-71,7

3.2. Monosaccharide composition of the polysaccharide complex in coltsfoot leaves

Total polysaccharide and free sugars content in terms of glucose (determined by spectrophotometric procedure with picric acid) should be not less than 10% according to State Pharmacopoeia of the Russian Federation XIV edition (SPRF XIV) [13].

Researchers from the Agricultural University of Norway have determined the composition and content of monosaccharides in polysaccharide complexes in leaves of *Tussilago farfara* L. (folia *Tussilaginis farfarae*). The predominant monosaccharides are GalUA (2-67%), Gal (11-25%), Glu (5-66%), Ara (8-37%). To a lesser extent, the polysaccharide complex of coltsfoot leaves includes Xyl (1-11%), ribose (Rib) (1-2%), Rha (4-7%), O-methylated sugars (1-2%). The high content of galacturonic acid can be explained by the presence of pectin substances. Pectin substances formed a large amount of free monosaccharide after hydrolysis. Researchers used commercial coltsfoot leaf samples (Norsk Medisinaldepot, Oslo). The polysaccharides were extracted with hot water. The crude polysaccharides were chromatographically fractionated on a DEAE-C column. The determination of monosaccharide composition and content was carried out by electrophoresis. The content of uronic acids was determined by the carbazole method. Neutral sugars were hydrolyzed with 2 N H_2SO_4. Further quantitative analysis was carried out using paper chromatography with aniline phthalate [22].

The monomer composition of water-soluble polysaccharides from purified coltsfoot leaves was determined by Fourier to transform infrared spectroscopy, Raman spectroscopy, and cellulose acetate electrophoresis. The presence of Gal, Xyl, glucuronic and galacturonic acids was determined by electrophoresis. Spectroscopic methods additionally showed the presence of Man [23].

Researchers at the Siberian State Medical University (Tomsk) determined the composition and content of monomeric residues of polysaccharide complexes of coltsfoot leaves. They isolated fractions of galactoarabinanes, rhamnans, rhamnogalactones, etc. The first fraction contained Gal (46.41%), Ara (38.08%), Rha (11.90%), Man (2.59%), Xyl (1, 02%), the second - Gal (29.58%), Ara (24.86%), Rha (19.98%), Man (17.48%), Glu (8.11%), the third - Gal (53.24%), Glu (21.81%), Ara (14.49%), Man (6.14%), Rha (4.33%), the fourth - Rha (44.16%), Gal (30.63%), GalUA (25.20%), fifth - Rha (100%), sixth - Gal (57.62%), Rha (27.02%), Glu (8.26%), Ara (7.1%). Leaves were collected in the Tomsk region and Altai Territory. The ratio of components is shown in Table 2. The extraction was carried out with hydrochloric acid concentrated in purified water. The ratio between the plant material and the extractant was 1:20. The separation was performed on a DEAE cellulose column. The uronic acid content was determined using spectrophotometry by reaction with carbazole. Hydrolysis of polysaccharides was carried out with 2M TFA. The obtained monosaccharides were derivatized and analyzed using gas chromatography with a mass selective detector [24]. The monosaccharide profile of the polysaccharide complex of coltsfoot leaves is shown in Table 3.

Table 2: Composition and ratio of monosaccharides of fractions of water-soluble polysaccharides

Fraction number	Monomer composition	Ratio
1	Gal:Ara:Rha	3,9:3,2:1
2	Gal:Ara:Rha:Man:Glu	3,6:3,1:2,5:2,2:1
3	Gal:Glu:Ara	3,7:1,5:1,0
4	Rha:Gal:GalUA	1,75:1,2:1,0
5	Rha	-
6	Gal:Rha	2:1

Table 3: Monosaccharide composition of the polysaccharide complex in coltsfoot leaves

Monosaccharide	Content, %
Galactose (Gal)	11-57,62
Glucose (Glu)	5-66
Arabinose (Ara)	7,1-38,08
Galacturonic acid (GalUA)	2-67
Xylose (Xyl)	1-11
Rhamnose (Rha)	4-100
Mannose (Man)	2,59-17,48
Ribose (Rib)	1-2

3.3. Monosaccharide composition of the polysaccharide complex in licorice roots

Carbohydrate content in licorice roots varies in 11.8-23.3 % [25]. Data on the monosaccharide composition of licorice roots (Radices Glycyrrhizae) presented in the literature is insufficient because the main research is devoted to the study of glycyrrhizin and its derivatives. Glycyrrhizin (saponin) is believed to exhibit the main pharmacological effect of licorice roots.

However, N. Wittschier with colleagues (Institute of Pharmaceutical Biology and Chemistry, Munich, Germany) determined the composition and content of monosaccharides in polysaccharide fractions. They studied the effect of polysaccharides of licorice root on inhibition of adhesion of H. pylori to the gastric mucosa. The prevailing licorice root monosaccharides are glucuronic acid (GluUA) (18.8%), Glu (16.5%), Gal (14.9%), Ara (11.5). Licorice roots also contain Man (6.9%), GalUA (5.9%), Rha (3.2%), Rib (2.1%), Xyl (1.1%), fucose (Fuc) (0.9%) in minor amounts. Licorice root samples were obtained from Caesar & Loretz (Hilden, Germany). Triple extraction of plant material was performed with water at 8 ℃ for 20 hours. The crude polysaccharides were fractionated chromatographically on a DEAE column by gradient elution with deionized water, sodium phosphate buffer, and 0.05 N sodium hydroxide. The determination of the total content of uronic acids was carried out according to the method of Blumenkrantz and Asboe-Hansen with o-hydroxy diphenyl. Hydrolysis of polysaccharides was carried out with 2M TFA. Quantitative analysis of monosaccharides was performed by ion-exchange HPLC with an amperometric detector with a CarboPac ™ column, a protective column, and a BorateTrap ™ column. Elution was performed with water and sodium hydroxide in gradient mode [26]. Thus, we can assume the presence of glucuronans, galacturonans, arabinogalacturonans in the licorice roots.

There is also data on the monosaccharide composition of polysaccharide fractions obtained by separation on a DEAE column of Ural licorice roots. The percentage of each component of the fractions was determined by Fourier-transform IR spectrometry. All fractions were heteropolysaccharides and contained Glu, Gal, Ara, and Rha residues. Researchers at Central Southern University (Changsha, China) found that the crude polysaccharide complex obtained during the experiment contained 6.98% uronic acids, Glu (26.14%), Gal (22.4%), Ara (7.58%) Rha

(0.76%), Man (1.13%). The neutral polysaccharide fraction contained Glu (23.4%), Gal (25.18%), Ara (8.32%). Acid polysaccharide fractions included uronic acids (6.12-28.01%), Glu (1.13-14%), Gal (22.04-25.67%), Ara (17.54-31.44 %), Rha (0.61-3.36%), Man (0.68-1.95%). All fractions were heteropolysaccharides and had residues of Glu, Gal, Ara, and Rha. Ural licorice samples were obtained from Yanchi (Ningxia Province, China). Plant material was extracted three times with 95% ethanol to remove lipid components. Further extraction was carried out with hot water (1: 9) at 80 ℃ for one hour. Fractionation was performed on a DEAE-52 flow column. Three polysaccharide fractions were obtained after elution with deionized water and NaCl aqueous solution (0.1, 0.25, 0.5, and 1M), dialysis, and concentration. Hydrolysis of polysaccharides was performed using 2M TFA. A quantitative analysis of monosaccharides of Ural licorice roots was carried out by GC-MS [27].

The data on the monosaccharide profile of the polysaccharide complex of licorice roots are summarized in Table 4.

Table 4: Monosaccharide composition of the polysaccharide complex of licorice roots (Radices *Glycyrrhizae*)

Monosaccharide	Content, %
Arabinose (Ara)	7,58-31,44
Glucose (Glu)	1,13-26,14
Galactose (Gal)	14,9-25,67
Glucuronic acid (GluUA)	18,8
Galacturonic acid (GalUA)	5,9
Mannose (Man)	0,68-6,9
Rhamnose (Rha)	0,61-3,36
Ribose (Rib)	2,1
Xylose (Xyl)	1,1
Fucose (Fuc)	0,9

3.4. Monosaccharide composition of the polysaccharide complex in Pectorales species No 2

Information on the monomer composition of polysaccharide complexes obtained during the review study is summarized in Table 5. The composition and content of monosaccharides in Pectorales species No 2 are given in Table 6.

Table 5: Composition and ratio of monosaccharides of polysaccharide complexes of plantain leaves, coltsfoot leaves and licorice roots.

Polysaccharide complexes	Monosaccharides, ratio
Licorice roots (Radices Glycirrhizae)	
Glucuronans; galactouronans; arabinogalactouronans; uronic acids; proteoglycans.	Fucose 0,9%, rhamnose 3,2%, arabinose 11,5%, galactose 14,9%, glucose 16,2 %, mannose 6,9%, ribose 2,1%, xylose 1,1%, galacturonic acid 5,9%, glucuronic acid 18,8%. PSH-1 (with protein): glucose (26,14%), galactose (22,4%), arabinose (7,58%), rhamnose (0,76%), mannose (0,13%). PSH-2: glucose (23,4%), galactose (25,18%), arabinose (8,32%) PSH-3: glucose (14%), galactose (25,67%), arabinose (17,54%), rhamnose (0,61%), mannose (0,68%). PSH-4: glucose (1,13%), galactose (22,04%), arabinose (31,44%) rhamnose (3,36%), mannose (1,95%).
Coltsfoot leaves (Folia Tussilaginis farfarae)	
Arabinogalactans; rhamnogalactouronans; rhamnans; galactorhamnans.	PSH 1: galactose, arabinose, rhamnose (3,9:3,2:1) PSH 2: galactose, arabinose, rhamnose, манноза, glucose (3,6:3,1:2,5:2,2:1) PSH 3: galactose, glucose, arabinose (3,7:1,5:1) PSH 4: rhamnose, galactose, D-galacturonic acid (1,75:1,2:1,0) PSH 5: rhamnose PSH 6: galactose, rhamnose (2:1)
Plantain leaves (Folia plantaginis majoris)	
Rhamnogalactouronans; galactans; arabinogalactans; xylogalactouronans; pectins.	arabinose 8,8-37,36% glucose 41,3-53,8% mannose 11,7-36,25% galactose 8-46,11% rhamnose 3,3-16,96% xylose 11,45-22,22% galacturonic acid 36,93-71,7%

Table 6: Estimated (approximate) monosaccharide composition of the polysaccharide complex PS No 2.

Monosaccharide	Approximate content,%
Arabinose (Ara)	2,27-15,23
Glucose (Glu)	0,34-26,40
Galactose (Gal)	2,4-23,0
Mannose (Man)	0,20-10,87
Rhamnose (Rha)	0,18-40
Xylose (Xyl)	0,33-6,66
Ribose (Rib)	0,4-0,8
Galacturonic acid (GalUA)	0,8-26,8
Glucuronic acid (GluUa)	5,64
Fucose (Fuc)	0,27

CONCLUSION

The modern pharmaceutical market of expectorant drugs provides consumers with synthetic medicines, herbal medicines, and multi-component drugs. The trend in the use of herbal medicines is growing over time. Herbal medicines have an important place in the treatment of bronchopulmonary diseases. Today plantain leaves, coltsfoot leaves, licorice roots and PS No 2 are often prescribed by general practitioners due to the high safety profile of herbal drugs and centuries of experience in the use of them. Polysaccharides are one of the groups of biologically active compounds with expectorant and anti-inflammatory activity. The structure and type of bond of monosaccharides cause a variation in the structure of polysaccharides of the above-mentioned plants. An analysis of the literature data made it possible to understand the monomer composition of the polysaccharide complexes of the plantain and coltsfoot leaves and licorice roots. This data will help to predict which monosaccharides prevail in the carbohydrate component of each medicinal plant material in PS No 2. The main monosaccharides of plantain leaves are galactose, arabinose, galacturonic acid. The polysaccharide complex of coltsfoot leaves consists mainly of galactose, glucose, and galacturonic acid. Licorice roots are rich in glucose, galactose, and arabinose.

Thus, the study of polysaccharide monomers will help to better understand the chemical profile of these biologically active compounds. This study makes it possible to systematize the scientific base that makes it possible to reasonably carry out standardization of a multicomponent herbal drug [28].

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CONFLICTS OF INTEREST

None.

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